FILE 'REGISTRY' ENTERED AT 13:33:29 ON 10 JUN 2003

=> S CELLULASE/CN
L1 1 CELLULASE/CN

=> D

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS
RN 9012-54-8 REGISTRY
CN ***Cellulase (9CI)*** (CA INDEX NAME)
OTHER NAMES:
CN .beta.-1,4-D-Endoglucanase
CN .beta.-1,4-Endoglucan hydrolase
CN .beta.-1,4-Glucanase
CN .beta.-1,4-Glycanase
CN 1,4-.beta.-D-Endoglucanase
CN 1,4-.beta.-D-Glucan 4-glucanohydrolase
CN 1,4-.beta.-D-Glucan endoglucanase
CN 1,4-.beta.-Glycanase
CN 800 NSK
CN 800NSK
CN Acremozyme
CN Alkali cellulase
CN AUS 0301
CN Auxilase
CN Avicelase
CN Avicelase I
CN Bactosol JA
CN Bactosol JN
CN Biocellulase
CN Biocellulase A
CN Biocellulase ZK
CN Biosoft
CN Biotouch C 25
CN Biotouch L
CN Carezyme
CN Carezyme 1000L
CN Carezyme 4500L
CN Cease
CN Cellodextrinase
CN Cellsoft L
CN Celluclast
CN Celluclast 1.5 LFG
CN Celluclast 1.5L
CN Celluclast 1.5LFG
CN Celluclast 2.0L
CN Celluclast 250L
CN Celluclast 2L
CN Cellulase 2322
CN Cellulase A
CN Cellulase A 3
CN Cellulase A Amano 3
CN Cellulase Cx
CN Cellulase EG III
CN Cellulase F
CN Cellulase H
CN Cellulase K 344
CN Cellulase K 425
CN Cellulase ML
CN Cellulase Ononzuka 3S
CN Cellulase T Amano 4
ADDITIONAL NAMES NOT AVAILABLE IN THIS FORMAT - Use FCN, FIDE, or ALL for DISPLAY
DR 9037-40-5, 145172-25-4, 143296-48-4, 152443-09-9, 149718-64-9, 160995-61-9, 179530-34-8, 214975-89-0
MF Unspecified
CI COM, MAN
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSCHEM, CSNB, DDFU, DRUGU, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, MSDS-OHS, NAPRALERT, NIOSHTIC, PIRA, PROMT, RTECS*, TOXCENTER, USAN,

```
      USPAT2, USPATFULL, VTB
        (*File contains numerically searchable property data)
     Other Sources:   DSL**, EINECS**, TSCA**
        (**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
           13374 REFERENCES IN FILE CA (1957 TO DATE)
             162 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
           13380 REFERENCES IN FILE CAPLUS (1957 TO DATE)


FILE 'CAPLUS' ENTERED AT 13:34:01 ON 10 JUN 2003


=> S CELLULASE OR L1
         15882 CELLULASE
          3405 CELLULASES
         16443 CELLULASE
                 (CELLULASE OR CELLULASES)
         13383 L1
L2       17506 CELLULASE OR L1

=> S THERMOSTABLE;S THERMAL TOLERANT
L3       10452 THERMOSTABLE


        883483 THERMAL
            61 THERMALS
        883509 THERMAL
                 (THERMAL OR THERMALS)
         21281 TOLERANT
            29 TOLERANTS
         21286 TOLERANT
                 (TOLERANT OR TOLERANTS)
L4           12 THERMAL TOLERANT
                 (THERMAL(W)TOLERANT)

=> S ACIDOTHERMUS;S CELLULOLYTICUS;S GUXA OR GUX(W)A;S GH6 OR GH(W)6;S GH12 OR GH(W)12

L5           60 ACIDOTHERMUS


L6          153 CELLULOLYTICUS


             2 GUXA
             7 GUX
      17119823 A
             0 GUX(W)A
L7           2 GUXA OR GUX(W)A


            10 GH6
         20772 GH
           541 GHS
         20976 GH
                 (GH OR GHS)
       3286315 6
            30 GH(W)6
L8           40 GH6 OR GH(W)6


             4 GH12
         20772 GH
           541 GHS
         20976 GH
                 (GH OR GHS)
       1205238 12
            14 GH(W)12
L9           18 GH12 OR GH(W)12
```

```
=> S CBDII OR CBD(W)II;S CBDIII OR CBD(W)III
             2 CBDII
          1179 CBD
           123 CBDS
          1205 CBD
                 (CBD OR CBDS)
       1919692 II
           700 IIS
       1920092 II
                 (II OR IIS)
             4 CBD(W)II
L10          5 CBDII OR CBD(W)II


             1 CBDIII
          1179 CBD
           123 CBDS
          1205 CBD
                 (CBD OR CBDS)
        943237 III
           190 IIIS
        943331 III
                 (III OR IIIS)
             0 CBD(W)III
L11          1 CBDIII OR CBD(W)III

=> S L2 AND (L2,L3)
L12      17506 L2 AND ((L2 OR L3))

=> S L12 AND L5
L13         43 L12 AND L5

=> S L5(W)L6
L14         53 L5(W)L6

=> S L14 AND L12
L15         41 L14 AND L12

=> S L7 AND L13
L16          2 L7 AND L13

=> S L13 NOT L16
L17         41 L13 NOT L16

=> D L16 1-2 CBIB ABS;D L17 1-41 CBIB ABS

L16  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2003 ACS
2003:435217  Thermal tolerant  ***cellulase***  from  ***acidothermus***
     cellulolyticus.  Ding, Shi-You; Adney, William S.; Vinzant, Todd B.;
     Himmel, Michael E.; Decker, Stephen R. (USA).  U.S. Pat. Appl. Publ. US
     20030104522 A1 20030605, 20 pp.  (English).  CODEN: USXXCO.  APPLICATION:
     US 2001-917383 20010728.
AB   The invention provides a thermal tolerant  ***cellulase***  that is a
     member of the glycoside hydrolase family.  The invention further discloses
     this  ***cellulase***  as  ***GuxA***  .  ***GuxA***  has been
     isolated and characterized from  ***Acidothermus***  cellulolyticus.
     The invention further provides recombinant forms of the identified
      ***GuxA***  .  Methods of making and using  ***GuxA***  polypeptides,
     including fusions, variants, and derivatives, are also disclosed.

L16  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2003 ACS
2003:118016  Document No. 138:165733  Sequences of an  ***Acidothermus***
     cellulolyticus  ***thermostable***  ***cellulase***  ***GuxA***
     and use as detergent.  Ding, Shi-you; Adney, William S.; Vinzant, Todd B.;
     Himmel, Michael E.; Decker, Stephen R. (Midwest Research Institute, USA).
     PCT Int. Appl. WO 2003012109 A1 20030213, 47 pp.  DESIGNATED STATES: W:
     AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO,
     CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, ES, FI, FI, GB, GD, GE,
     GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
     LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM,
```

AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US23817 20010728.

AB The invention provides protein and DNA sequences of a novel ***Acidothermus*** cellulolyticus thermal tolerant (***thermostable***) ***cellulase*** ***GuxA*** that is a member of the glycoside hydrolase family. The invention further provides recombinant forms of the identified ***GuxA*** . Methods of making and using ***GuxA*** polypeptides, including fusions, variants, and derivs., are also disclosed. The invention further relates to the use of ***GuxA*** in making detergents and degradating agricultural biomass.

L17 ANSWER 1 OF 41 CAPLUS COPYRIGHT 2003 ACS

2003:396366 Thermal tolerant exoglucanase from ***acidothermus*** cellulolyticus. Adney, William S.; Ding, Shi-You; Vinzant, Todd B.; Himmel, Michael E.; Decker, Stephen R.; McCarter, Suzanne Lantz (USA). U.S. Pat. Appl. Publ. US 20030096342 A1 20030522, 20 pp. (English). CODEN: USXXCO. APPLICATION: US 2001-917384 20010728.

AB The invention provides a thermal tolerant ***cellulase*** that is a member of the glycoside hydrolase family. The invention further discloses this ***cellulase*** as Gux1. Gux1 has been isolated and characterized from ***Acidothermus*** cellulolyticus.The invention further provides recombinant forms of the identified Gux1. Methods of making and using Gux1 polypeptides, including fusions, variants, and derivatives, are also disclosed.

L17 ANSWER 2 OF 41 CAPLUS COPYRIGHT 2003 ACS

2003:118001 Document No. 138:165728 Sequences of an ***Acidothermus*** cellulolyticus ***thermostable*** avicelase AviIII and use as detergent. Ding, Shi-You; Adney, William S.; Vinzant, Todd B.; Himmel, Michael E. (Midwest Research Institute, USA). PCT Int. Appl. WO 2003012090 A2 20030213, 44 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US23818 20010728.

AB The invention provides protein and DNA sequences of a novel ***Acidothermus*** cellulolyticus thermal tolerant (***thermostable***) ***cellulase*** AviIII that is a member of the glycoside hydrolase family. The invention further provides recombinant forms of the identified AviIII. Methods of making and using AviIII polypeptides, including fusions, variants, and derivs., are also disclosed. The invention further relates to the use of AviIII in making detergents.

L17 ANSWER 3 OF 41 CAPLUS COPYRIGHT 2003 ACS

2003:114969 Expression and import of an active ***cellulase*** from a thermophilic bacterium into the chloroplast both in vitro and in vivo. Jin, Rongguan; Richter, Stefan; Zhong, Rong; Lamppa, Gayle K. (Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, 60637, USA). Plant Molecular Biology, 51(4), 493-507 (English) 2003. CODEN: PMBIDB. ISSN: 0167-4412. Publisher: Kluwer Academic Publishers.

AB A bacterial ***thermostable*** ***cellulase*** , the endo-1,4-.beta.-D-glucanase E1 from ***Acidothermus*** cellulolyticus, was imported into chloroplasts, and an active enzyme was recovered both in vitro and in vivo. Precursor fusion proteins were synthesized with E1 or its catalytic domain, CD, fused to the transit peptide of ferredoxin or ribulose-bisphosphate carboxylase activase for stromal targeting. A spacer region of 1, 5 or 15 amino acids was included carboxy to the transit peptide. The efficiency of import and processing by the stromal processing peptidase depended on the nature of the transit peptide and the passenger protein, and increased with the length of the spacer between them. Besides finding E1 or CD in the stroma, protein was arrested in the envelope during import showing that structural features of E1 and CD,

along with their proximity to the transit peptide, influence translocation. The cellulose binding domain and/or serine/proline/threoline-rich linker of E1 may impede efficient import. Significantly, most precursors for E1 and CD synthesized by in vitro translation possessed endoglucanse activity that was temp.-dependent, and required the residues AGGGY at the N-terminus of E1 and CD. Furthermore, activity was detected upon import into chloroplasts. Based on the in vitro analyses, five precursor fusion proteins were selected to det. if E1 and CD would be successfully targeted to chloroplasts in vivo. In transgenic tobacco plants, E1 and CD accumulated in both the stromal and membrane fractions and, importantly, chloroplast exts. showed endoglucanase activity.

L17 ANSWER 4 OF 41 CAPLUS COPYRIGHT 2003 ACS

2002:649217 Document No. 137:313398 Development of a flexible system for the simultaneous conversion of biomass to industrial chemicals and the production of industrial biocatalysts. Gao, Johnway; Hooker, Brian S.; Skeen, Rodney S.; Anderson, Daniel B. (Environmental Technology Division, Pacific Northwest National Laboratory, Richland, WA, 99352, USA). ACS Symposium Series, 823(Advancing Sustainability through Green Chemistry and Engineering), 145-161 (English) 2002. CODEN: ACSMC8. ISSN: 0097-6156. Publisher: American Chemical Society.

AB A flexible system was developed for the simultaneous conversion of biomass (waste starch and agricultural and food-processing wastes) to industrial chems., industrial biocatalysts, and single-cell proteins (as animal feeds). In particular, the expression of a bacterial enzyme, .beta.-glucuronidase (GUS), was investigated using a genetically modified starch-degrading Saccharomyces strain in suspension cultures in starch media. Different sources of starch, including corn and waste potato starch, were used for yeast biomass accumulation and GUS expression studies under controls of inducible and constitutive promoters. A ***thermostable*** bacterial ***cellulase*** (E1 .beta.-1,4-endoglucanase) gene of ***Acidothermus*** cellulolyticus was also cloned into an episomal plasmid expression vector and expressed in the starch-degrading Saccharomyces strain.

L17 ANSWER 5 OF 41 CAPLUS COPYRIGHT 2003 ACS

2002:332354 Document No. 136:351397 Transgenic plants expressing ligninase and ***cellulase*** for degradation of lignin and cellulose to produce sugars. Sticklen, Masomeh B.; Dale, Bruce E.; Maqbool, Shahina (Michigan State University, USA). PCT Int. Appl. WO 2002034926 A2 20020502, 126 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US32538 20011018. PRIORITY: US 2000-PV242408 20001020.

AB This invention provides a transgenic plant expressing ligninase and ***cellulase*** genes from microbes operably linked to a DNA encoding a signal peptide which targets the fusion polypeptide produced therefrom to an organelle of the plant, in particular the chloroplasts. When the transgenic plants are harvested, the plants are ground to release the ligninase and ***cellulase*** which then degrade the lignin and cellulose of the transgenic plants to produce the fermentable sugars. Furthermore, the sugar can be used in fermn. of ethanols.

L17 ANSWER 6 OF 41 CAPLUS COPYRIGHT 2003 ACS

2002:323635 Document No. 137:43353 Effect of single active-site cleft mutation on product specificity in a ***thermostable*** bacterial ***cellulase*** . Rignall, Tauna R.; Baker, John O.; McCarter, Suzanne L.; Adney, William S.; Vinzant, Todd B.; Decker, Stephen R.; Himmel, Michael E. (Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 98-100(Biotechnology for Fuels and Chemicals), 383-394 (English) 2002. CODEN: ABIBDL. ISSN: 0273-2289. Publisher: Humana Press Inc..

AB Mutation of a single active-site cleft tyrosyl residue to a glycyl residue significantly changes the mixt. of products released from phosphoric

acids-wollen cellulose (PSC) by EIcd, the catalytic domain of the endoglucanase-I from ***Acidothermus*** cellulolyticus. The percentage of glucose in the product stream is almost 40% greater for the Y245G mutant (and for an addnl. double mutant, Y245G/Q204A) than for the wild type enzyme. Comparisons of results for digestion PSC and of pretreated yellow poplar suggest that the obsd. shifts in product specificity are connected to the hydrolysis of a more easily digestible fraction of both substrates. A model is presented that relates the changes in product specificity to a mutation-driven shift in indexing of the polymeric substrate along the extended binding-site cleft.

L17 ANSWER 7 OF 41 CAPLUS COPYRIGHT 2003 ACS

2002:323625 Document No. 137:43352 Exploration of cellulose surface-binding properties of ***Acidothermus*** cellulolyticus Cel5A by site-specific mutagenesis. McCarter, Suzanne L.; Adney, William S.; Vinzant, Todd B.; Jennings, Edward; Eddy, Fannie Posey; Decker, Stephen R.; Baker, John O.; Sakon, Joshua; Himmel, Michael E. (Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 98-100(Biotechnology for Fuels and Chemicals), 273-287 (English) 2002. CODEN: ABIBDL. ISSN: 0273-2289. Publisher: Humana Press Inc..

AB Understanding the interactions between ***cellulases*** and cellulosic substrates is crit. to the development of an efficient artificial ***cellulase*** system for conversion of biomass to sugars. We directed specific mutations to the interactive surface of the ***Acidothermus*** cellulolyticus EI endoglucanase catalytic domain. The cellulose-binding domain is not translated in these mutants. Amino acid mutations were designed either to change the surface charge of the protein or to modify the potential for hydrogen bonding with cellulose. The relationship between ***cellulase*** -to-cellulose (Avicel PH101) binding and hydrolysis activity was detd. for various groupings of mutations. While a significant increase in hydrolysis activity was not obsd., certain clusters of residues did significantly alter substrate binding and some interesting correlations emerged. In the future, these observations may be used to aid the design of endoglucanases with improved performance on pretreated biomass.

L17 ANSWER 8 OF 41 CAPLUS COPYRIGHT 2003 ACS

2002:186412 Improved ***cellulases*** through protein engineering. Himmel, Michael E.; Brady, John W.; Sakon, Joshua; Adney, William S.; McCarter, Suzanne; Decker, Stephen R.; Vinzant, Todd B.; Rignall, Tauna; Baker, John O.; Skopec, Cathy; Ding, Shi-You (National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA). Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002, CELL-002. American Chemical Society: Washington, D. C. (English) 2002. CODEN: 69CKQP.

AB A key objective to ensure the economic competitiveness of bioethanol with traditional fuels is to reduce ***cellulase*** cost. Alone, or in combination, increases in specific activity and thermal tolerance should reduce the amt. of enzyme necessary to achieve the required extent of cellulose conversion. We have demonstrated that a protein engineering approach could improve the cellulose degrading activity of ***Acidothermus*** cellulolyticus Cel5A, one component of a ternary ***cellulase*** mixt. which also includes Trichoderma reesei Cel7A and Aspergillus niger beta-D-glucosidase. We used site directed mutagenesis to produce Cel5Acd carrying specific mutations in the active site as well as a putative thermal tolerance-conferring residue. More recently, groups of EI surface amino acid mutations were designed to change the strength of its interaction with substrate. The results of testing these mutants combined with new understanding of cellulose surface chem. from mol. mechanics modeling illustrates the need for continued focus on studies probing the fundamental nature of ***cellulase*** action.

L17 ANSWER 9 OF 41 CAPLUS COPYRIGHT 2003 ACS

2002:144986 Document No. 136:290922 4-Methyl-7-thioumbelliferyl-.beta.-D-cellobioside: A Fluorescent, Nonhydrolyzable Substrate Analogue for ***Cellulases*** . Barr, Brian K.; Holewinski, Ronald J. (Department of Chemistry, Loyola College in Maryland, Baltimore, MD, 21210-2699, USA). Biochemistry, 41(13), 4447-4452 (English) 2002. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB The kinetics of cellulose binding and hydrolysis by ***cellulases***

is not well understood except at steady-state conditions. For use in studies of ***cellulase*** pre-steady-state and steady-state kinetics, we have prepd. 4-methyl-7-thioumbelliferyl-.beta.-D-cellobioside (MUS-CB), a ground-state nonhydrolyzable analog of the fluorescent ***cellulase*** substrate 4-methylumbelliferyl-.beta.-D-cellobioside (MU-CB). MUS-CB is not hydrolyzed by the catalytic domain of ***cellulase*** E1 from ***Acidothermus*** cellulolyticus under conditions where this enzyme rapidly degrades MU-CB. Thermodn. parameters describing the steady-state binding of MUS-CB to Thermobifida fusca ***cellulase*** Cel6A are similar to those for MU-CB, indicating that MUS-CB can be used in place of MU-CB to study binding events in the Cel6A active-site cleft. In the pre-steady-state, MUS-CB binds to Cel6A by a simple, one-step bimol. assocn. reaction. It is anticipated that similar thio-contg. 4-methylumbelliferyl compds. will have applications in studies of other enzyme systems.

L17 ANSWER 10 OF 41 CAPLUS COPYRIGHT 2003 ACS

2002:141758 Document No. 137:164308 Dramatic effects of truncation and sub-cellular targeting on the accumulation of recombinant microbial ***cellulase*** in tobacco. Ziegelhoffer, Thomas; Raasch, John A.; Austin-Phillips, Sandra (University of Wisconsin Biotechnology Center, University of Wisconsin-Madison, Madison, WI, 53706, USA). Molecular Breeding, 8(2), 147-158 (English) 2001. CODEN: MOBRFL. ISSN: 1380-3743. Publisher: Kluwer Academic Publishers.

AB The economical bioconversion of lignocellulosic biomass to ethanol is dependent on the availability of large quantities of inexpensive ***cellulase*** enzymes. One way to reduce the cost of such enzymes is to produce them in crop plants at high levels. In order to assess factors that limit recombinant ***cellulase*** expression in plants, we have introduced the gene encoding E1 endo-1,4-.beta.-glucanase (***cellulase***) of ***Acidothermus*** cellulolyticus into tobacco (Nicotiana tabacum) plants. Both the holoenzyme (E1) and catalytic domain (E1cd) were targeted to three sub-cellular compartments; the cytosol, chloroplast and apoplast. Accumulation of both E1 and E1cd was greatest in the apoplast, with levels more than 100-fold higher than obsd. for cytosolic accumulation. In all three compartments, E1cd accumulated to higher levels than the full-length enzyme. By combining truncation and apoplastic localization, an increase in expression of more than 500-fold was achieved, compared to cytosolic full-length E1. This effect is primarily post-transcriptional, since E1cd mRNA levels are very similar despite the range of E1cd accumulation obsd. Recombinant E1cd, expressed at up to 1.6% total sol. protein, is extremely stable in both crude leaf exts. and dried leaf material.

L17 ANSWER 11 OF 41 CAPLUS COPYRIGHT 2003 ACS

2001:229028 Document No. 134:248007 Downstream box variants for use in increasing the efficiency of translation of foreign genes in plastids. Chaudhuri, Sumita (Calgene LLC, USA). PCT Int. Appl. WO 2001021782 A2 20010329, 52 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US26052 20000922. PRIORITY: US 1999-PV156071 19990924.

AB Elements that can be used to increase the efficiency of translation of foreign genes on plastid ribosomes are described. Specifically, variants of the downstream box (DB) that lies 3' of the Shine-Dalgrano sequence and that is involved in interaction with the 16S rRNA in the ribosome are described. A series of variants of known downstream boxes were generated and tested for their effects on the level of expression of a bacterial gene (the .beta.-1,4-endoglucanase gene of ***Acidothermus*** E1) from a bacteriophage T7 promoter in tobacco plastids. A clear effect of the DB on the efficiency of translation was obsd.

L17 ANSWER 12 OF 41 CAPLUS COPYRIGHT 2003 ACS

2001:168160 Document No. 134:203422 Production of ***cellulase*** in plastids of transgenic plants, use of ferredoxin and rubisco activase transit peptides. Lamppa, Gayle (Arch Developement Corporation, USA). PCT Int. Appl. WO 2001016338 A2 20010308, 25 pp. DESIGNATED STATES: W:

AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-IB1214 20000831. PRIORITY: US 1999-388788 19990902.

AB The invention provides ***cellulase*** fusion proteins, which contain the transit peptide of ferredoxin or rubisco activase, linked to spacer amino acids, linked to .beta.-1-4-endoglucanase E1 from ***Acidothermus*** cellulolyticus. The invention relates that the fusion proteins can be cleaved by a stromal processing peptidase (SPP), and may contain the only a part of of .beta.-1-4-endoglucanase E1, such as the catalytic domain (CD). The invention also provides chimeric genes encoding said ***cellulase*** fusion proteins, constructs comprising cDNA mols. encoding said fusion proteins, and the use of said constructs in transforming plants for the recombinant prodn. of said ***cellulase*** . The invention relates that the transgenic plant accumulates ***cellulase*** in plastids, such as chloroplasts in leaves, and/or amyloplasts in storage tubers or roots. The invention, in the example section, presented the partial sequence of various ***cellulase*** fusion proteins, and characterized their ***cellulase*** activity and ability to be transported to chloroplasts.

L17 ANSWER 13 OF 41 CAPLUS COPYRIGHT 2003 ACS

2000:824394 Document No. 134:2062 ***Acidothermus*** cellulolyticus E1 endoglucanase variants Y245G, Y82R and W42R with increased catalytic activity. Himmel, Michael E.; Adney, William S.; Baker, John O.; Vinzant, Todd B.; Thomas, Steven R.; Sakon, Joshua; Decker, Stephen R. (Midwest Research Institute, USA). PCT Int. Appl. WO 2000070031 A1 20001123, 30 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US13971 20000519. PRIORITY: US 1999-PV134925 19990519.

AB The invention provides a method for making a glycosyl hydrolase characterized by an increase in catalytic activity on an insol. substrate. An active site assocd. glycosyl-stabilizing amino acid of the hydrolase is thus replaced with an amino acid, the replacing amino acid not strongly binding a disaccharide product in the active site. The method for making a glycosyl hydrolase characterized by an increase in catalytic activity on a sol. substrate comprises replacing a hydrophobic substrate binding amino acid of the hydrolase with a pos. charged amino acid. The invention specifically provides ***Acidothermus*** cellulolyticus E1 endoglucanase variants, comprising Y42R, W82R, or Y245G, and the DNA sequences encoding the enzymes. Ki values for inhibition of hydrolysis of 4-.beta.-D-cellobioside by native and Y245G mutant E1 indicate that the mutant catalytic domain binds cellobiose 15-fold less tightly than does the native enzyme, i.e., an increase in Ki from 2 to 30 mM cellobiose and a decrease in apparent binding energy of 1.7 kcal/mol.

L17 ANSWER 14 OF 41 CAPLUS COPYRIGHT 2003 ACS

2000:820588 Document No. 135:134757 Improved plant-based production of E1 endoglucanase using potato: Expression optimization and tissue targeting. Dai, Ziyu; Hooker, Brian S.; Anderson, Daniel B.; Thomas, Steven R. (Bioprocessing Group, Environmental Technology Division, Pacific Northwest National Laboratory, Richland, WA, 99352, USA). Molecular Breeding, 6(3), 277-285 (English) 2000. CODEN: MOBRFL. ISSN: 1380-3743. Publisher: Kluwer Academic Publishers.

AB Optimization of ***Acidothermus*** cellulolyticus endoglucanase (E1) gene expression in transgenic potato (Solanum tuberosum L.) was examd. in this study, where the E1 coding sequence was transcribed under control of a leaf specific promoter (tomato RbcS-3C) or the Mac promoter (a hybrid promoter of mannopine synthase promoter and cauliflower mosaic virus 35S promoter enhancer region). Av. E1 activity in leaf exts. of potato

transformants, in which E1 protein was targeted by a chloroplast signal peptide and an apoplast signal peptide were much higher than those by an E1 native signal peptide and a vacuole signal peptide. E1 protein accumulated up to 2.6% of total leaf sol. protein, where E1 gene was under control of the RbcS-3C promoter, alfalfa mosaic virus 5'-untranslated leader, and RbcS-2A signal peptide. E1 protein prodn., based on av. E1 activity and E1 protein accumlation in leaf exts., is higher in potato than those measured previously in transgenic tobacco bearing the same transgene constructs. Comparisons of E1 activity, protein accumulation, and relative mRNA levels showed that E1 expression under control of tomato RbcS-3C promoter was specifically localized in leaf tissues, while E1 gene was expressed in both leaf and tuber tissues under control of Mac promoter. This suggests dual-crop applications in which potato vines serve as enzyme prodn. "bioreactors" while tubers are preserved for culinary applications.

L17 ANSWER 15 OF 41 CAPLUS COPYRIGHT 2003 ACS
2000:779462 Document No. 134:112153 Molecular mechanics studies of ***cellulases*** . Palma, Rocio; Zuccato, Pierfrancesco; Himmel, Michael E.; Liang, Guyan; Brady, John W. (Department of Food Science, Cornell University, Ithaca, NY, 14853, USA). ACS Symposium Series, 769(Glycosyl Hydrolases for Biomass Conversion), 112-130 (English) 2000. CODEN: ACSMC8. ISSN: 0097-6156. Publisher: American Chemical Society.
AB Mol. mechanics (MM) simulations are ideally suited for studying the properties of aq. solns. of biol. mols., which are difficult to probe exptl. To date, there have been relatively few MM calcns. of ***cellulases*** , although there are a no. of questions which could perhaps be answered with modeling studies. Several examples of MM calcns. of ***cellulase*** systems are given to illustrate the potential applications in understanding and modifying ***cellulase*** activity. These include the calcn. of the change in substrate binding affinity for the T240F point mutant of the E1 ***cellulase*** from ***Acidothermus*** cellulolyticus, and the calcn. of the potential of mean force for the binding of a glucose mol. to methane, as a model for the side-chain of alanine.

L17 ANSWER 16 OF 41 CAPLUS COPYRIGHT 2003 ACS
2000:779459 Document No. 134:39546 Production of microbial ***cellulases*** in transgenic crop plants. Hooker, B. S.; Dai, Z.; Anderson, D. B.; Quesenberry, R. D.; Ruth, M. F.; Thomas, S. R. (Pacific Northwest National Laboratory, Richland, WA, 99352, USA). ACS Symposium Series, 769(Glycosyl Hydrolases for Biomass Conversion), 55-90 (English) 2000. CODEN: ACSMC8. ISSN: 0097-6156. Publisher: American Chemical Society.
AB The expression of ***Acidothermus*** cellulolyticus endoglucanase (E1) gene and Trichoderma reesei cellobiohydrolase (CBH1) gene in transgenic tobacco (Nicotiana tabaccum) and potato (Solanum tuberosum) was examd. in this study. CBH1 and E1 produced in transgenic tobacco plants were found to be biol. active and to accumulate in leaves at levels of up to 0.05% and 2.6% of total sol. protein, resp. The biochem. characteristics of plant-based recombinant E1 enzyme were similar to those of natural E1 purified from bacterial culture. In addn., plant-derived E1 was resistant to plant proteolysis at different developmental stages. Transgenic plants exhibited normal growth and developmental characteristics with photosynthetic rates similar to those of untransformed SR1 tobacco plants. Based on E1 activity and E1 protein accumulation in leaf exts., E1 expression in potato is much higher than that measured in transgenic tobacco bearing the same transgene constructs. E1 expression under the control of the RbcS-3C promoter was specifically localized in leaf tissues, while E1 was expressed in both leaf and tuber tissues under the control of the constitutive Mac promoter. This suggests dual-crop applications in which potato vines serve as enzyme prodn. "bioreactors" while tubers are preserved for culinary applications. Economic anal. demonstrated that the cost of large-scale E1 prodn., based on potato vine-based expression, could be as low as $1.40/kg enzyme.

L17 ANSWER 17 OF 41 CAPLUS COPYRIGHT 2003 ACS
2000:397829 Document No. 133:291695 Expression of ***Acidothermus*** cellulolyticus endoglucanase E1 in transgenic tobacco: biochemical characteristics and physiological effects. Dai, Ziyu; Hooker, Brian S.; Anderson, Daniel B.; Thomas, Steven R. (Pacific Northwest National

Laboratory, Richland, WA, 99352, USA). Transgenic Research, 9(1), 43-54 (English) 2000. CODEN: TRSEES. ISSN: 0962-8819. Publisher: Kluwer Academic Publishers.

AB The expression of the ***Acidothermus*** cellulolyticus endoglucanase E1 gene in transgenic tobacco (Nicotiana tabacum) was examd. in this study, where E1 coding sequence was transcribed under the control of a leaf-specific Rubisco small subunit promoter (tomato RbcS-3C). Targeting the E1 protein to the chloroplast was established using a chloroplast transit peptide of Rubisco small subunit protein (tomato RbcS-2A) and confirmed by immunocytochem. The E1 produced in transgenic tobacco plants was biol. active, and accumulated in leaves at levels of up to 1.35% of total sol. protein. Optimum temp. and pH for E1 enzyme activity in leaf exts. were 81.degree. and 5.25, resp. E1 activity remained const. on a gram fresh leaf wt. basis, but dramatically increased on a total leaf sol. protein basis as leaves aged, or when leaf disks were dehydrated. E1 protein in old leaves, or after 5 h dehydration, was partially degraded although E1 activity remained const. Transgenic plants exhibited normal growth and developmental characteristics with photosynthetic rates similar to those of untransformed SR1 tobacco plants. Results from these biochem. and physiol. analyses suggest that the chloroplast is a suitable cellular compartment for accumulation of the hydrolytic E1 enzyme.

L17 ANSWER 18 OF 41 CAPLUS COPYRIGHT 2003 ACS

2000:195089 Document No. 133:14662 Accumulation of a ***thermostable*** endo-1,4-.beta.-D-glucanase in the apoplast of Arabidopsis thaliana leaves. Ziegler, Matthew T.; Thomas, Steven R.; Danna, Kathleen J. (Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, 80309-0347, USA). Molecular Breeding, 6(1), 37-46 (English) 2000. CODEN: MOBRFL. ISSN: 1380-3743. Publisher: Kluwer Academic Publishers.

AB The catalytic domain of a ***thermostable*** (Topt = 81.degree.C) endo-1,4-.beta.-D-glucanase from the eubacterium, ***Acidothermus*** cellulolyticus, was expressed in the apoplast of tobacco BY-2 suspension cells and leaves of Arabidopsis thaliana plants. The apoplast-targeting cassette designed for this work consists of the cauliflower mosaic virus 35S promoter, the tobacco mosaic virus .OMEGA. translational enhancer, the sequence encoding the tobacco Pr1a signal peptide, and the polyadenylation signal of nopaline synthase. Recombinant E1 catalytic domain was targeted to the ER by the signal peptide and secreted into the apoplast via the default pathway. Secretion of the enzyme did not detectably affect the growth rate of transgenic BY-2 cells, although the protein was enzymically active at elevated temps. Similarly, transgenic plants exhibited no abnormal phenotypes correlating with expression of the enzyme. Close agreement between independent immunochem. and activity-based assays indicates that the enzyme accumulated to concns. up to 26% of the total sol. protein in leaves of primary A. thaliana transformants. The amt. of functional endoglucanase produced illustrates that plants can accumulate very large quantities of enzyme for com. biomass conversion.

L17 ANSWER 19 OF 41 CAPLUS COPYRIGHT 2003 ACS

2000:31397 Document No. 132:60128 Expression of enzymes involved in cellulose modification. Himmel, Michael E.; Schaaf, David J.; Stalker, David M.; Thomas, Steven R. (Calgene Llc, USA). U.S. US 6013860 A 20000111, 10 pp. (English). CODEN: USXXAM. APPLICATION: US 1998-122533 19980724.

AB Novel compns. and methods are provided useful for genetic engineering of plant cells to provide expression in the plastids of a plant or plant cell of cellulose-degrading enzymes. Thus, a thermophilic E1 ***cellulase*** from ***Acidothermus*** cellulolyticus is employed in constructs to direct expression from the plastid of plant cells. Furthermore, transplastomic tobacco plants expressing E1 ***cellulase*** demonstrate a high level of expression of the ***cellulase*** enzyme. Expressed ***cellulase*** can reduce the cellulose content in transgenic plants, thereby improving the digestibility of plant material.

L17 ANSWER 20 OF 41 CAPLUS COPYRIGHT 2003 ACS

1999:713405 Document No. 132:46933 Transgenic fungal-based conversion of waste starch to industrial enzymes. Gao, J.; Hooker, B. S.; Skeen, R. S.; Anderson, D. B. (Pacific Northwest National Laboratory, Bioprocessing Group, Richland, WA, 99352, USA). Biomass: A Growth Opportunity in Green Energy and Value-Added Products, Proceedings of the Biomass Conference of

the Americas, 4th, Oakland, Calif., Aug. 29-Sept. 2, 1999, Volume 1, 895-901. Editor(s): Overend, Ralph P.; Chornet, Esteban. Elsevier Science: Oxford, UK. (English) 1999. CODEN: 68IQAG.

AB The prodn. of a bacterial enzyme, beta-glucuronidase (GUS), was investigated using a genetically modified starch-degrading Saccharomyces strain in suspension cultures of various waste starch sources. A shuttle plasmid expression vector was constructed using a yeast episomal plasmid. The glucuronidase (gus) gene was placed under the control of an inducible promoter, GAL1, and terminated by a transcription terminator, Tcycl. Different sources of starches including corn and waste potato starch were used for yeast biomass accumulation and glucuronidase expression studies. In addn., a ***thermostable*** bacterial ***cellulase*** , ***Acidothermus*** cellulolyticus E1 endoglucanase was cloned into the plasmid expression vector and expressed in the starch-degrading Saccharomyces strain.

L17 ANSWER 21 OF 41 CAPLUS COPYRIGHT 2003 ACS

1999:91360 Molecular mechanics studies of ***cellulases*** . Brady, John W.; Palma, Rocio; Skopec, Catherine; Himmel, Michael E. (Department of Food Science, Cornell University, Ithaca, NY, 14853, USA). Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25, CELL-031. American Chemical Society: Washington, D. C. (English) 1999. CODEN: 67GHA6.

AB Examples of Mol. Mechanics calcns. of ***cellulases*** and related systems will be discussed. Potentials of mean force for the binding of glucose mols. to amino acid side chains can be calcd. using umbrella sampling in Mol. Dynamics simulations. Similar free energy profiles can be calcd. for bringing glucose into the binding site of an enzyme. Thermodn. cycle free energy perturbation calcns. can also be used to compute differences in binding affinities for different substrates or mutants. Mol. docking and conformational energy calcns. can be used to model substrate chains into catalytic binding sites and to monitor conformational changes in the enzymes upon substrate binding. Similar approaches can be used to place binding domains onto cellulose surfaces, or in the case of E1 from ***Acidothermus*** cellulolvticus, the catalvtic domain can be directly docked on the microcryst. cellulose surface.

L17 ANSWER 22 OF 41 CAPLUS COPYRIGHT 2003 ACS

1999:91216 Catalytically enhanced ***cellulase*** . Lovett, R. M.; Sakon, J. (Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, 72701, USA). Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25, CARB-056. American Chemical Society: Washington, D. C. (English) 1999. CODEN: 67GHA6.

AB The designed mutation from Tyr245 to Gly (Y245G) in the endocellulase E1 from ***Acidothermus*** cellulolyticus indeed enhanced its enzymic capabilities by 12.+-.1 %. The mutant was discovered through examg. the crystal structure of wild-type enzyme/substrate complex which clarified the enzyme mechanism at at. level and identified that Tyr245 as a key residue to interact with a leaving group. Four mutants at the site were generated and screened for higher activity against cellotriose, identifying the mutant Y245G. To examine the cause of the enhanced mutant Y245G, its crystal structure was solved and was refined at 2.5 .ANG. resoln. to an R-factor of 0.19 (R-free = 0.24). The loss of Tyr245 side chain allowed Pro246 to adopt an energetically favorable conformation by 1.5 kcal/mol, subsequently it caused significant structural changes to the residue Gln247 that interact with a glucosyl unit in a leaving group. The loss of the platform as well as a hydrogen bond invariably, resulted in 15kcal/mol less binding interactions with the product and explains the increased enzymic activity resulting from the loss in product inhibition.

L17 ANSWER 23 OF 41 CAPLUS COPYRIGHT 2003 ACS

1998:645719 Document No. 130:1580 The use of capillary viscometry, reducing end-group analysis, and size exclusion chromatography combined with multi-angle laser light scattering to characterize endo-1,4-.beta.-D-glucanases on carboxymethylcellulose: a comparative evaluation of the three methods. Vlasenko, Elena Yu; Ryan, Anya I.; Shoemaker, Charles F.; Shoemaker, Sharon P. (California Institute of Food and Agricultural Research, Davis, CA, 95616, USA). Enzyme and Microbial Technology, 23(6), 350-359 (English) 1998. CODEN: EMTED2. ISSN: 0141-0229. Publisher: Elsevier Science Inc..

AB Three methods were used to characterize seven purified endo-1,4-.beta.-D-glucanases derived from different microbial sources (Trichoderma reesei, Thermomonospora fusca, and ***Acidothermus*** cellulolyticus) on CM-cellulose (CMC). The methods included capillary viscometry, reducing end-group anal., and high performance size exclusion chromatog. combined with multi-angle laser light scattering (HPSEC-MALLS). The investigation was performed with the objective of comparative evaluation of the different methods for characterizing endoglucanases, particularly in relation to their endo- and exomode of action. The measurement of the initial rate of reducing end-group formation using disodium 2,2'-bicinchoninate (BCA) was found to be the most accurate method for detn. of endoglucanase activity. The BCA method was highly sensitive, simple to perform, and directly gave the no. of bonds broken, thus allowing for expression of endoglucanase activity in IU (.mu.mol of .beta.-1,4-glucosidic bonds hydrolyzed in 1 min during the initial period of hydrolysis). The viscometric method was simple to perform and highly sensitive for the internal bonds cleaved, but did not account for the hydrolysis of CMC near the chain end, and thus only allowed for expression of endoglucanase activity in arbitrary viscometric units. The HPSEC-MALLS technique provided the no.-av. mol. wt. (Mn) of CMC, thus allowing the quantification of the no. of the bonds broken during degrdn. of CMC; however, reproducibility of the method was low, esp. for the high-mol. wt. fragments of CMC at the beginning of hydrolysis. As hydrolysis proceeded to the more advanced stages, the HPSEC-MALLS method gave an overestimated (compared to the reducing end-group anal.) values for Mn, probably due to insufficient sensitivity of the light-scattering detector for the low-mol. wt. products of CMC degrdn. The combined use of the three methods allowed characterization of endoglucanases according to their selectivity for hydrolysis of internal bonds within a CMC mol. which was expressed as the ratio of the initial rate of viscosity decrease to the initial rate of glucosidic bonds broken. This ratio was found to be unique for each endoglucanase, and, therefore, no universal equation could be established for all endoglucanases for conversion of arbitrary viscometric units to IU of activity.

L17 ANSWER 24 OF 41 CAPLUS COPYRIGHT 2003 ACS

1998:351011 Document No. 129:5741 Hydrolysis of cellulose using ternary mixtures of purified ***cellulases*** . Baker, John O.; Ehrman, Christine I.; Adney, William S.; Thomas, Steven R.; Himmel, Michael E. (Biotechnology Center for Fuels and Chemicals, National Renewable Energy Laboratory, Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 70-72, 395-403 (English) 1998. CODEN: ABIBDL. ISSN: 0273-2289. Publisher: Humana Press Inc..

AB The saccharification of microcryst. cellulose by reconstituted ternary mixts. of purified ***cellulases*** (one endoglucanase and two cello-bio-hydrolases) was studied over the entire range of mixt. compns. Ternary plots are used to compare the performance of 5 synthetic mixts. drawn from the ***cellulase*** systems of ***Acidothermus*** cellulolyticus, Trichoderma reesei, Thermomonospora fusca, and Thermotoga neapolitana. At least 1 synthetic mixt. using enzymes from 3 different organisms delivered performance competitive with that of a "native", i.e., co-evolved, ternary system drawn exclusively from T. reesei. This heterologous system, consisting of the endoglucanase E1 from A. cellulolyticus and the exoglucanases CBHI from T. reesei and E3 from T. fusca, is forgiving from the system-design point of view, in that it delivers high saccharification rates over a wide range of mixt. compns.

L17 ANSWER 25 OF 41 CAPLUS COPYRIGHT 2003 ACS

1998:76000 Document No. 128:151117 Improved thermostability in ***cellulase*** by production of the C-terminal truncated catalytic domain. Adney, William S.; Thomas, Steven R.; Baker, John O.; Himmel, Michael E.; Chou, Yat-Chen (Midwest Research Institute, USA). U.S. US 5712142 A 19980127, 19 pp., Cont.-in-part of U.S. 5,536,655. (English). CODEN: USXXAM. APPLICATION: US 1996-604913 19960222. PRIORITY: US 1989-412434 19890926; US 1992-826089 19920127; US 1993-125115 19930921; US 1994-276213 19940715.

AB The gene encoding ***Acidothermus*** cellulolyticus E1 endoglucanase is cloned and expressed in Pichia pastoris. A new modified E1 endoglucanase enzyme comprising the catalytic domain (residues 1-358) of the full-size, mature E1 enzyme demonstrates enhanced thermostability and is produced by 2 methods. The first method of producing the new modified

E1 is proteolytic cleavage to remove the cellulose binding domain and linker peptide of the full size E1. The second method of producing the new modified E1 is genetic truncation of the gene encoding the full size E1 so that the catalytic domain is expressed in the expression product.

L17 ANSWER 26 OF 41 CAPLUS COPYRIGHT 2003 ACS
1996:467404 Document No. 125:107081 Gene encoding the E1 endoglucanase from ***Acidothermus*** cellulolyticus. Thomas, Steven R.; Laymon, Robert A.; Himmel, Michael E. (Midwest Research Institute, USA). U.S. US 5536655 A 19960716, 21 pp., Cont.-in-part of U.S. 5, 366, 884. (English). CODEN: USXXAM. APPLICATION: US 1994-276213 19940715. PRIORITY: US 1989-412434 19890926; US 1992-826089 19920127; US 1993-125115 19930921.
AB The gene encoding ***Acidothermus*** cellulolyticus E1 endoglucanase is cloned, sequenced, and expressed in heterologous microorganisms by std. recombinant DNA techniques. The 3004-bp sequence encodes a 562-amino acid precursor enzyme contg. a 41-residue signal sequence which is cleaved to yield the active E1 endoglucanase enzyme. New modified E1 endoglucanase enzymes can be produced by std. techniques of mutagenesis and mixed domain construction. The E1 endoglucanase is useful for hydrolyzing cellulose to sugars for simultaneous or later fermn. into alc.

L17 ANSWER 27 OF 41 CAPLUS COPYRIGHT 2003 ACS
1996:457926 Document No. 125:108737 Crystal structure of ***thermostable*** family 5 endocellulase E1 from ***Acidothermus*** cellulolyticus in complex with cellotetraose. Sakon, Joshua; Adney, William S.; Himmel, Michael E.; Thomas, Steven R.; Karplus, P. Andrew (Section of Biochemistry Molecular and Cell Biology, Cornell University, Ithaca, NY, 14853, USA). Biochemistry, 35(33), 10648-10660 (English) 1996. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.
AB The crystal structure of the catalytic domain (cd) of ***thermostable*** ***cellulase*** E1 from A. cellulolyticus in complex with cellotetraose was solved by multiple isomorphous replacement and refined at 2.4 .ANG. resoln. to an R-factor of 0.18 (Rfree = 0.24). E1cd is a member of the 4/7 superfamily of hydrolases, and as expected, its structure was an (.alpha./.beta.)8 barrel, which constitutes a prototype for family 5/subfamily 1 ***cellulases*** . The cellotetraose mol. bound in a manner consistent with the expected Michaelis complex for the glycosylation half-reaction and revealed that all 8 residues conserved in family 5 enzymes were involved in recognition of the glycosyl group attacked during cleavage. Whereas only 3 residues are conserved in the whole 4/7 superfamily (the Asn/Glu duo and the Glu from which the name is derived), structural comparisons showed that all 8 residues conserved in family 5 had functional equiv. in the other 4/7 superfamily members, strengthening the case that mechanistic details are conserved throughout the superfamily. On the basis of the structure, a detailed sequence of phys. steps of the cleavage mechanism is proposed. A close approach of 2 key Glu residues provides an elegant mechanism for the shift in the pKa of the acid/base for the glycosylation and deglycosylation half-reactions. Finally, purely structure-based comparisons were used to show that significant differences exist in structural similarity scores resulting from different methods and suggested that caution should be exercised in interpreting such results in terms of implied evolutionary relations.

L17 ANSWER 28 OF 41 CAPLUS COPYRIGHT 2003 ACS
1996:333025 Document No. 125:2981 Cloning of ***cellulase*** genes from ***Acidothermus*** cellulolyticus. Lastick, Stanley M.; Tucker, Melvin P.; Grohmann, Karel (Midwest Research Institute, USA). U.S. US 5514584 A 19960507, 12 pp., Cont.-in-part of U.S. Ser. No. 74, 369, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1994-266930 19940627. PRIORITY: US 1993-74369 19930611.
AB A process is described for moving fragments that code for ***cellulase*** activity from the genome of A. cellulolyticus to several plasmid vectors and the subsequent expression of active ***cellulase*** activity in E. coli.

L17 ANSWER 29 OF 41 CAPLUS COPYRIGHT 2003 ACS
1996:262842 Document No. 124:309585 Gene coding for the E1 endoglucanase from ***Acidothermus*** cellulolyticus. Thomas, Steven R.; Laymon, Robert A.; Himmel, Michael E. (Midwest Research Institute, USA). PCT Int. Appl. WO 9602551 A1 19960201, 33 pp. DESIGNATED STATES: W: AU, BR, CA,

CN, DE, ES, GB, JP, KP, KR, NZ, SE; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US8868 19950714. PRIORITY: US 1994-276213 19940715.

AB The gene encoding ***Acidothermus*** cellulolyticus E1 endoglucanase was cloned and expressed in heterologous microorganisms by std. recombinant DNA techniques. The 3004-bp fragment of DNA contains a coding portion of 1686 bp corresponding to a deduced protein sequence of 562 amino acids and contg. a 41-residue signal moiety. The gene architecture is similar to that of ***cellulase*** genes isolated from other actinomycete bacteria. New modified E1 endoglucanase enzymes are produced along with variants of the gene and enzyme. The E1 endoglucanase is useful for hydrolyzing cellulose to sugars for simultaneous or later fermn. into alc. Recombinant prodn. (e.g., in Escherichia coli or Saccharomyces lividans) result in much improved rate of of enzyme prodn., thereby lowering the cost of ***cellulase*** and the prodn. of alc. using cellulosic materials as substrate.

L17 ANSWER 30 OF 41 CAPLUS COPYRIGHT 2003 ACS

1996:11558 Document No. 124:49197 Synergism between purified bacterial and fungal ***cellulases*** . Baker, John O.; Adney, William S.; Thomas, Steven R.; Nieves, Rafael A.; Chou, Yat-Chen; Vinzant, Todd B.; Tucker, Melvin P.; Laymon, Robert A.; Himmel, Michael E. (Alternative Fuels Div., National Renewable Energy Laboratory, Golden, CO, 80401-3393, USA). ACS Symposium Series, 618(Enzymatic Degradation of Insoluble Carbohydrates), 113-41 (English) 1995. CODEN: ACSMC8. ISSN: 0097-6156. Publisher: American Chemical Society.

AB A standardized comparative study measured glucose release and synergistic effects in the solubilization of microcryst. cellulose by binary mixts. of 11 fungal and bacterial ***cellulases*** (eight endoglucanases and three exoglucanases). Evaluation of 16 endo/exo pairs revealed that bacterial/fungal hybrid pairs are very effective in solubilizing microcryst. cellulose. Of nine bacterial/fungal hybrid pairs studied, six were ranked among the nine most synergistic combinations, and six bacterial/fungal pairs were also among the top nine pairs in terms of sol.-sugar release. One hybrid pair (***Acidothermus*** cellulolyticus E1 and Trichoderma reesei CBH I) was ranked first in both synergism and sugar-release. In exo/exo synergism expts., the performance of Thermomonospora fusca E3 confirmed its mode of action as "CBH II-like" (i.e., E3 is synergistic with T. reesei CBH I but not with T. reesei CBH II). Studies of endo/endo interactions suggested a possible means of categorizing endoglucanases in terms of substrate specificity.

L17 ANSWER 31 OF 41 CAPLUS COPYRIGHT 2003 ACS

1995:556109 Document No. 123:4072 Quantitation of ***Acidothermus*** cellulolyticus E1 endoglucanase and Thermomonospora fusca E3 exoglucanase using enzyme-linked immunosorbent assay (ELISA). Nieves, Rafael A.; Chou, Yat-Chen; Himmel, Michael E.; Thomas, Steven R. (Appl. Biol. Sci. Branch, Natl. Renewable Energy Lab., Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 51/52, 211-23 (English) 1995. CODEN: ABIBDL. ISSN: 0273-2289. Publisher: Humana.

AB Two distinct quant. indirect ELISAs were developed to det. the concn. of recombinant ***cellulase*** components in culture filtrates. A monoclonal antibody (E1P7) was used as the primary antibody in developing an ELISA specific for A. cellulolyticus E1 ***cellulase*** (endoglucanase) (I). Likewise, a polyclonal rabbit serum (Ab684) was used to develop an ELISA specific for Thermomonospora fusca E3 exocellobiohydrolase (exoglucanase) (II). Dose-response curves indicated a dynamic range for both assays between 0.01 and 0.08 .mu.g/mL (1-8 ng/assay) when purified enzymes were used as stds. These ELISAs were used to est. the concns. of secreted recombinant I and/or II in culture supernatants of Streptomyces lividans strain TK24 in which the corresponding genes were cloned and expressed.

L17 ANSWER 32 OF 41 CAPLUS COPYRIGHT 2003 ACS

1994:321433 Document No. 120:321433 A new ***thermostable*** endoglucanase, ***Acidothermus*** cellulolyticus E1. Synergism with Trichoderma reesei CBH I and comparison to Thermomonospora fusca E5. Baker, John O.; Adney, William S.; Nieves, Rafael A.; Thomas, Steven R.; Wilson, David B.; Himmel, Michael E. (Alternat. Fuels Div., Natl. Renewable Energy Lab., Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 45-46, 245-56 (English) 1994. CODEN: ABIBDL. ISSN:

0273-2289.

AB A new ***thermostable*** endoglucanase, ***Acidothermus*** cellulolyticus E1, and another bacterial endoglucanase, E5 from Thermomonospora fusca, each exhibit striking synergism with a fungal cellobiohydrolase (Trichoderma reesei CBH I) in the saccharification of microcryst. cellulose. In either case did the ratio of endoglucanase to exoglucanase that demonstrated max. synergism coincide exactly with the ratio that actually released the max. quantity of sol. sugar for a given total ***cellulase*** loading. The difference between the two ratios, after significant hydrolysis of the substrate, was considerably larger in the case of A. cellulolyticus E1. For both endoglucanase pairings with CBH I, the offset between the ratio for max. synergism and the ratio of maximal sol. sugar prodn. was found to be a function of digestion time.

L17 ANSWER 33 OF 41 CAPLUS COPYRIGHT 2003 ACS

1994:100558 Document No. 120:100558 ***Thermostable*** endoglucanase from ***Acidothermus*** cellulolyticus. Himmel, Michael E.; Adney, William S.; Tucker, Melvin P.; Grohmann, Karel (Midwest Research Institute, USA). U.S. US 5275944 A 19940104, 10 pp. Cont.-in-part of the U.S. 5,110,735. (English). CODEN: USXXAM. APPLICATION: US 1992-826089 19920127. PRIORITY: US 1989-412434 19890926.

AB A purified low mol. wt. ***cellulase*** endoglucanase I having a mol. wt. of between about 57,420 to about 74,580 daltons from ***Acidothermus*** cellulolyticus (ATCC 43068) is described. The ***cellulase*** is water sol., possesses both C1 and Cx types of enzyme activity, a high degree of stability toward heat, and exhibits optimum temp. activity at about 83.degree. at pH's from about 2 to about 9, and an inactivation temp. of about 110.degree. at pH's from about 2 to about 9.

L17 ANSWER 34 OF 41 CAPLUS COPYRIGHT 2003 ACS

1993:534386 Document No. 119:134386 ***Thermostable*** purified endoglucanases from thermophilic bacterium ***Acidothermus*** cellulolyticus. Himmel, Michael E.; Adney, William S.; Tucker, Melvin P.; Grohmann, Karel (Midwest Research Institute, USA). PCT Int. Appl. WO 9315186 A1 19930805, 26 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1993-US706 19930126. PRIORITY: US 1992-826089 19920127.

AB A low mol. wt. endoglucanase I from the ***cellulase*** complex of A. cellulolyticus and a method for prepg. the enzyme comprising ultrafiltration and column chromatog. are claimed. The enzyme has a mol. wt. of 57,420-74,580 Da, is water-sol., possesses both C1 and Cx activities, and exhibits high thermal stability. At pH 2-9, the optimal activity temp. is 83.degree. and the inactivation temp. is 110.degree..

L17 ANSWER 35 OF 41 CAPLUS COPYRIGHT 2003 ACS

1991:581469 Document No. 115:181469 ***Cellulase*** production by ***Acidothermus*** cellulolyticus: growth on Solka Floc cellulose and simple sugar mixtures. Shiang, Ming; Linden, James C.; Mohagheghi, Ali; Tucker, Melvin P.; Grohmann, Karel; Himmel, Michael E. (Dep. Microbiol., Colorado State Univ., Fort Collins, CO, 80523, USA). Biotechnology and Applied Biochemistry, 14(1), 30-40 (English) 1991. CODEN: BABIEC. ISSN: 0885-4513.

AB The effects of various sugar and N sources on ***cellulase*** prodn. by A. cellulolyticus grown on Solka Floc cellulose were examd. Solka Floc at concns. of 5-20 g/L, mixed with simple sugars at 2.5 g/L, were used as C sources. ***Cellulase*** activity was produced from the late log phase of growth and enzyme productivity was max. for fermns. using 15 g/L Solka Floc with several sugars. The substrate system using sucrose and Solka Floc produced the highest ***cellulase*** activity in the shortest fermn. time yet reported for A. cellulolyticus (0.110 U/mL in 50 h). Of the sugars added to Solka Floc cellulose fermns., D-cellobiose, D-glucose, D-fructose, D-xylose, and sucrose stimulated cellulolytic enzyme prodn., but only D-cellobiose and D-xylose were effective ***cellulase*** inducers. Different N sources significantly influenced the culture growth kinetics and enzyme prodn. The best prodn. of ***cellulase*** was with NH4Cl.

L17 ANSWER 36 OF 41 CAPLUS COPYRIGHT 2003 ACS

1991:557057 Document No. 115:157057 Production of ***cellulase*** by ***Acidothermus*** cellulolyticus. Shiang, Ming (Colorado State Univ., Fort Collins, CO, USA). 174 pp. Avail. Univ. Microfilms Int., Order No. DA9117209 From: Diss. Abstr. Int. B 1991, 52(1), 59 (English) 1990.
AB Unavailable

L17 ANSWER 37 OF 41 CAPLUS COPYRIGHT 2003 ACS
1991:468159 Document No. 115:68159 Regulation of ***cellulase*** synthesis in ***Acidothermus*** cellulolyticus. Shiang, Ming; Linden, James C.; Mohagheghi, Ali; Grohmann, Karel; Himmel, Michael E. (Dep. Microbiol., Colorado State Univ., Fort Collins, CO, 80523, USA). Biotechnology Progress, 7(4), 315-22 (English) 1991. CODEN: BIPRET. ISSN: 8756-7938.
AB The regulation of ***cellulase*** synthesis by induction and catabolite repression in the thermophilic, aerobic bacterium A. cellulolyticus was studied by using batch fermns. Various compds., such as L-sorbose, cAMP, L-glucose, 2-deoxyglucose (2-DG), glucose 1-phosphate (G-1-P), sophorose, salicin, sugar alcs., and iso-Pr thioglucoside (IPTGlu), were added along with Solka Floc to improve extracellular ***cellulase*** formation by the culture. When cAMP was added exogenously to A. cellulolyticus cultures in the concn. range of 0.01-0.2 g/L, cAMP did not affect cell growth; however, ***cellulase*** yields were increased with increasing levels of cAMP. The enzyme prodn. rates with the different levels of cAMP addn. during Solka-Floc fermns. were identical. L-Sorbose, L-glucose, 2-DG, G-1-P, sophorose, IPTGlu, and sugar alcs. enhanced ***cellulase*** activity produced in the medium, but the starting time and the time required to reach the max. enzyme activity were different in each condition. All these substances may function as moderators of cellulose synthesis. Only, cellobiose, xylose, sophorose, and unknown sol. derivs. from cellulose were considered as inducers. In a possible regulatory mechanism of ***cellulase*** synthesis, the repressor, inducer, cAMP, and moderator may be all involved in controlling the rate and the yield of enzyme prodn.

L17 ANSWER 38 OF 41 CAPLUS COPYRIGHT 2003 ACS
1991:467518 Document No. 115:67518 ***Thermostable*** endoglucanases from ***Acidothermus*** cellulolyticus. Mohagheghi, Ali; Tucker, Melvin P.; Himmel, Michael E.; Grohmann, Karel (Midwest Research Institute, USA). PCT Int. Appl. WO 9105039 A1 19910418, 26 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, RO, SD, SE, SU, US; RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, IT, LU, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1990-US4868 19900827. PRIORITY: US 1989-412434 19890926.
AB The ***thermostable*** endoglucanases (I) having C1 and Cx type enzymic activities are purified from thermophilic A. cellulolyticus cultures. I have optimal temps. of 70-80.degree. and comprise enzymes having mol wts. of 156,000-203,400 and 57,420-74,580 and 50000-70000 dalton. A. cellulolyticus was grown in a medium contg. yeast ext., salts, etc. for 20-36 h at 55.degree. to obtain a culture supernatant of 0.02 international filter paper units/mL. Purifn. protocols for I were given.

L17 ANSWER 39 OF 41 CAPLUS COPYRIGHT 2003 ACS
1991:183818 Document No. 114:183818 Enhanced production of ***cellulase*** using ***Acidothermus*** cellulolyticus in fed-batch culture. Shiang, Ming; Linden, James C.; Mohagheghi, Ali; Grohmann, Karel; Himmel, Michael E. (Dep. Microbiol., Colorado State Univ., Fort Collins, CO, 80523, USA). Applied Microbiology and Biotechnology, 34(5), 591-7 (English) 1991. CODEN: AMBIDG. ISSN: 0175-7598.
AB Fed-batch fermns. of A. cellulolyticus utilizing mixts. of cellulose and sugars were investigated for potential improvements in ***cellulase*** prodn. In these fermns., cellulose from several sources was combined with various simple sugars at selected concns. The best source of cellulose for ***cellulase*** prodn. was ball-milled Solka Floc at 15 g/L. Fed-batch fermns. with cellobiose and Solka Floc increased cell mass only slightly, but succeeded in significantly enhancing ***cellulase*** synthesis compared to batch conditions. Max. ***cellulase*** activities obtained from fermns. initiated with 2.5 g cellobiose/L and 15 g Solka Floc/L were 0.187 units (U)/mL, achieved by continuous feeding to maintain <0.1 g cellobiose/L, and 0.215 U/mL using the same initial medium when 2.5 g cellobiose/L was step-fed after the sugar was nearly consumed.

In batch dual-substrate systems consisting of simple sugars with Solka Floc, substrate inhibition was evident in terms of sp. growth rates, sp. productivity values, and max. enzyme yields. Limiting concns. of glucose or sucrose at 5 g/L and cellobiose at 2.5 g/L in the presence of Solka Floc yielded ***cellulase*** activities of 0.134, 0.159, and 0.164 U/mL, resp.

L17 ANSWER 40 OF 41 CAPLUS COPYRIGHT 2003 ACS
1990:457399 Document No. 113:57399 ***Cellulase*** production by ***Acidothermus*** cellulolyticus. Shiang, M.; Linden, J. C.; Mohagheghi, A.; Rivard, C. J.; Grohmann, K.; Himmel, M. E. (Dep. Microbiol., Colorado State Univ., Fort Collins, CO, 80523, USA). Applied Biochemistry and Biotechnology, 24-25, 223-35 (English) 1990. CODEN: ABIBDL. ISSN: 0273-2289.

AB A. cellulolyticus, an isolate from hot springs at Yellowstone National Park, produced ***cellulase*** enzyme when grown in cellobiose-contg. medium. The ***cellulase*** prodn., cell growth, and cellobiose degrdn. rates of batch culture in 2.5, 5.0, 7.5, and 10.0 g/L of cellobiose as a substrate were studied. The sp. growth rates were measured, and the .mu.max and Ks values based on these data were 0.2/h and 0.3 g cellobiose/L using the Monod equation. The max. ***cellulase*** activities (21-69 U/L) and volumetric productivities (between 0.92 and 2.49 U/L-h) were proportional to the concn. of cellobiose. Greatest ***cellulase*** prodn. in batch culture was achieved by use of secondary cellulosic substrates. Mixed substrate systems consisting of 5 g/L cellobiose and various Avicel concns. (5, 10, and 16 g/L) were also studied in batch culture. The max. ***cellulase*** activities were 53, 62, and 78 U/L, resp. The enzyme prodn. rate could be related to Avicel using the Monod equation. Here, max. volumetric productivity, Vmax, was 2.15 U/L-h and Ks was 7.5 g Avicel/L. Another mixed substrate system, consisting of 5 g/L cellobiose and 15 g/L Solka Floc, produced a max. ***cellulase*** concn. of 105 U/L.

L17 ANSWER 41 OF 41 CAPLUS COPYRIGHT 2003 ACS
1989:529547 Document No. 111:129547 Ultra- ***thermostable*** ***cellulases*** from ***Acidothermus*** cellulolyticus: comparison of temperature optima with previously reported ***cellulases*** . Tucker, Melvin P.; Mohagheghi, Ali; Grohmann, Karel; Himmel, Michael E. (Sol. Fuels Res. Div., Sol. Energy Res. Inst., Golden, CO, 80401, USA). Bio/Technology, 7(8), 817-20 (English) 1989. CODEN: BTCHDA. ISSN: 0733-222X.

AB The recent discovery of A. cellulolyticus genus novum, species novum, ATCC 43068, a moderately thermophilic, aerobic, cellulolytic bacterium in wood samples recovered from the acidic hot springs of northern Yellowstone National Park, Wyoming, affirms the notion that hitherto unknown microflora exist in nature in areas of extreme environment. The filter paper degrading enzymes (***cellulases***) produced by this new bacterium possess the highest temp. tolerance reported to date. The significance of this finding lies in the moderate temp., by comparison, for optimal cell growth required by the ***Acidothermus*** microorganism and in the potential for industrial application of the thermotolerant ***cellulase*** enzymes it produces.

```
=> S L2 AND (L3,L4)
L18         256 L2 AND ((L3 OR L4))

=> S L18 AND L5
L19          16 L18 AND L5

=> S L19 NOT L13
L20           0 L19 NOT L13

=> E DING S/AU
=> S E3,E15,E97,E98
            24 "DING S"/AU
           130 "DING S Y"/AU
            12 "DING SHI YOU"/AU
             2 "DING SHI YU"/AU
L21         168 ("DING S"/AU OR "DING S Y"/AU OR "DING SHI YOU"/AU OR "DING SHI
                YU"/AU)
```

```
=> E ADNEY W/AU
=> S E4-E6
             8 "ADNEY W S"/AU
             1 "ADNEY WILLIAM"/AU
            47 "ADNEY WILLIAM S"/AU
L22         56 ("ADNEY W S"/AU OR "ADNEY WILLIAM"/AU OR "ADNEY WILLIAM S"/AU)

=> E VINZANT T/AU
=> S E4,E5
             3 "VINZANT T B"/AU
            27 "VINZANT TODD B"/AU
L23         30 ("VINZANT T B"/AU OR "VINZANT TODD B"/AU)

=> E HIMMEL M/AU
=> S E3-E7
            10 "HIMMEL M"/AU
            31 "HIMMEL M E"/AU
             4 "HIMMEL MICHAEL"/AU
            95 "HIMMEL MICHAEL E"/AU
             1 "HIMMEL MICHAEL EDWARD"/AU
L24        141 ("HIMMEL M"/AU OR "HIMMEL M E"/AU OR "HIMMEL MICHAEL"/AU OR
               "HIMMEL MICHAEL E"/AU OR "HIMMEL MICHAEL EDWARD"/AU)

=> E DECKER S/AU
=> S E3,E9,E27,E28,E31,E32,E34-E36
            20 "DECKER S"/AU
             2 "DECKER S R"/AU
             6 "DECKER STEPHAN"/AU
             2 "DECKER STEPHEN"/AU
            16 "DECKER STEPHEN R"/AU
             1 "DECKER STEPHEN ROBERT"/AU
             1 "DECKER STEVE"/AU
             1 "DECKER STEVE R"/AU
             1 "DECKER STEVEN"/AU
L25         50 ("DECKER S"/AU OR "DECKER S R"/AU OR "DECKER STEPHAN"/AU OR
               "DECKER STEPHEN"/AU OR "DECKER STEPHEN R"/AU OR "DECKER STEPHEN
               ROBERT"/AU OR "DECKER STEVE"/AU OR "DECKER STEVE R"/AU OR "DECKE
               R STEVEN"/AU)

=> S L21,L22,L23,L24,L25
L26        335 (L21 OR L22 OR L23 OR L24 OR L25)

=> S L26 AND L2
L27         67 L26 AND L2

=> S L27 NOT L13
L28         40 L27 NOT L13

=> D 1-40 TI

=> S L26 AND L5
L29         33 L26 AND L5

=> S L29 NOT (L28,L13)
L30          6 L29 NOT ((L28 OR L13))

=> D 1-6 CBIB ABS

L30  ANSWER 1 OF 6  CAPLUS  COPYRIGHT 2003 ACS
2003:118017   Document No. 138:165734  Sequences of an   ***Acidothermus***
     cellulolyticus thermostable mannanase A (ManA) and use as detergent.
       ***Ding, Shi-You***  ;   ***Adney, William S.***  ;   ***Vinzant, Todd***
  ***      B.***  ;   ***Himmel, Michael E.***   (Midwest Research Institute, USA).
     PCT Int. Appl. WO 2003012110 A1 20030213, 46 pp.  DESIGNATED STATES: W:
     AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO,
     CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, ES, FI, FI, GB, GD, GE,
     GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
     LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM,
     AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM,
     CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT,
```

SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US23819 20010728.

AB The invention provides protein and DNA sequences of a novel ***Acidothermus*** cellulolyticus thermal tolerant (thermostable) mannanase A that is a member of the glycoside hydrolase family. The invention further provides recombinant forms of the identified ManA. Methods of making and using ManA polypeptides, including fusions, variants, and derivs., are also disclosed. Methods of using mannanase A, including for the processing of food and for use in food stuffs as bulking agents and the like, are also disclosed.

L30 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2003 ACS

2003:118004 Document No. 138:165730 Sequences of an ***Acidothermus*** cellulolyticus thermostable exoglucanase Guxl and use as detergent. ***Adney, William S.*** ; ***Ding, Shi-You*** ; ***Vinzant, Todd*** *** B.*** ; ***Himmel, Michael E.*** ; ***Decker, Stephen R.*** ; Lantz McCarter, Suzanne (Midwest Research Institute, USA). PCT Int. Appl. WO 2003012095 A1 20030213, 44 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US23820 20010728.

AB The invention provides protein and DNA sequences of a novel ***Acidothermus*** cellulolyticus thermal tolerant (thermostable) exoglucanase Guxl that is a member of the glycoside hydrolase family. The invention further provides recombinant forms of the identified Guxl. Methods of making and using Guxl polypeptides, including fusions, variants, and derivs., are also disclosed. The invention further relates to the use of Guxl in making detergents and degradating agricultural biomass.

L30 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2003 ACS

1995:739010 Document No. 123:192372 Purification and characterization of a low molecular weight thermostable .beta.-D-glucosidase from ***Acidothermus*** cellulolyticus. ***Himmel, Michael E.*** ; Tucker, Melvin P.; ***Adney, William S.*** ; Nieves, Rafael A. (Midwest Research Institute, USA). U.S. US 5432075 A 19950711, 9 pp. Cont.-in-part of U.S. 5,366,884. (English). CODEN: USXXAM. APPLICATION: US 1994-275995 19940715. PRIORITY: US 1989-412434 19890926; US 1992-826089 19920127; US 1993-125115 19930921.

AB A low mol. wt. .beta.-D-glucosidase produced from ***Acidothermus*** cellulolyticus ATCC 43068 has been purified and characterized. A procedure for purifn. of the enzyme is detailed. The enzyme is water sol., possesses activity against pNP-.beta.-D-glucopyranoside, has a high of degree of stability toward heat, exhibits optimal temp. activity at about 65.degree.C at a pH range of from about 2 to about 7, has an inactivation temp. of about 80.degree.C at a pH range of from about 2 to about 7, and has a mol. wt. of about 50.5-54.5 kDa as detd. by SDS-PAGE.

L30 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2003 ACS

1995:487322 Document No. 122:259452 Low molecular weight thermostable .beta.-D-glucosidase from ***Acidothermus*** cellulolyticus. ***Adney, W. S.*** ; Tucker, M. P.; Nieves, R. A.; Thomas, S. R.; ***Himmel, M. E.*** (Applied Biol. Sci. Section, Alternative Fuels Div., Golden, CO, 80401, USA). Biotechnology Letters, 17(1), 49-54 (English) 1995. CODEN: BILED3. ISSN: 0141-5492. Publisher: Chapman and Hall.

AB A new thermostable .beta.-glucosidase (I) was isolated from A. cellulolyticus culture broth. I had a mol. wt. of 52,500, a pI of 4.1, and a pH activity optimum of 4.5. The optimum temp. for activity on p-nitrophenyl-.beta.-D-glucoside was 63.degree.. This value was .apprx.20.degree. higher than temp. optima displayed by most previously characterized .beta.-glucosidases.

L30 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2003 ACS

1995:278405 Document No. 122:75491 Thermostable endoglucanase II of ***Acidothermus*** cellulolyticus and method for its purification. ***Adney, William S.*** ; Thomas, Steven R.; Nieves, Rafael A.;

Himmel, Michael E. (Midwest Research Institute, USA). U.S. US 5366884 A 19941122, 15 pp. Cont.-in-part of U.S. 5,275,944. (English). CODEN: USXXAM. APPLICATION: US 1993-125115 19930921. PRIORITY: US 1989-412434 19890926; US 1992-826089 19920127.

AB A purified low mol. wt. endoglucanase II from ***Acidothermus*** cellulolyticus (ATCC 43068) is disclosed. The endoglucanase is water sol., possesses both C1, and Cx types of enzyme activity, a high degree of stability toward heat, and exhibits optimum temp. activity at about 81.degree. at pH's from about 2 to about 9, and at a inactivation temp. of about 100.degree. at pH's from about 2 to about 9. A process for prepg. the enzyme comprises concg. the A. cellulolyticus culture broth by using ammonium sulfate pptn. (between 40% and 60% satd. solns.) or by ultrafiltration using an Amicon ultrafiltration app. equipped with PM-10 membranes. The conc. can be stored at -20.degree. in the presence of 20% glycerol for periods greater than a year with no loss in enzyme activity. The high mol. wt. endoglucanase complex produced by this process has a temp. optimum near 65.degree.. Endoglucanase II (and endoglucanase I) are prepd. from the complex by high-performance size-exclusion and ion exchange column chromatog.

L30 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2003 ACS

1986:511610 Document No. 105:111610 Isolation and characterization of ***Acidothermus*** cellulolyticus gen. nov., sp. nov., a new genus of thermophilic, acidophilic, cellulolytic bacteria. Mohagheghi, A.; Grohmann, K.; ***Himmel, M.*** ; Leighton, L.; Updegraff, D. M. (Sol. Energy Res. Inst., Colorado Sch. Mines, Golden, CO, 80401, USA). International Journal of Systematic Bacteriology, 36(3), 435-43 (English) 1986. CODEN: IJSBA8. ISSN: 0020-7713.

AB Twelve isolates of thermophilic, acidophilic, cellulolytic bacteria were obtained from 3 different primary enrichment cultures from acidic hot springs at Yellowstone National Park, Wyo. The 3 isolates which had the highest cellulolytic activity, as shown by the diam. of clearing zones surrounding colonies on cellulose agar plates, were selected for intensive study. All were gram-variable, nonsporulating aerobic rods which formed no pigment. They grew at 37-65.degree., with optimum growth at 55.degree.. The pH range for growth was 3.5-7, with an optimum pH of 5. The guanine-plus-cytosine content of the DNA was .apprx.60.7 mol%. The organisms are resistant to penicillin G at 100 .mu.g/mL. They share several important features with Thermus strains, namely heterotrophic, aerobic, and thermophilic mode of growth; morphol. features; sensitivity to lysozyme; and presence of catalase. They differ in other important aspects, such as the pattern of C sources utilized for growth, the pH and temp. profiles of growth, the pattern of sensitivity to antibiotics, the guanine-plus-cytosine content of DNA, the compn. of amino acids in the cell walls, and the structure of the cell walls. Thermus Species are very sensitive to penicillin G, whereas the studied strains are resistant. The studied strains also are different, in important respects from the genus Thermomicrobium. Therefore, ***Acidothermus*** cellulolyticus gen. nov., sp. nov., was designated.